THE

CEREBELLAR CORTEX OF THE DOG.

By HENRY J. BERKLEY, M.D.

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The investigation was undertaken to determine if the use of some of the newer methods of hardening and staining, in connection with the old, together with a collective view of a large number of sections and teasings differently treated, would not lighten to some extent the obscurity now existing concerning the fine anatomy of this, the most complex organ of the central nervous system, or of the entire body.

The limits of the paper will not enable me to more than touch occasionally upon the works of others (Deiters, Koschewnikoff, Hadlich, Schwalbe, Dennissenko, Beevor, Obersteiner, etc.), nor to enter at length upon the discussion of disputed points: it is simply a *résumé* of what I have myself seen in the course of a series of examinations.

Dogs, cats and pigeons were at first used, but the cerebellum of the first-named animal presented so many points of superiority over the others, both in the tissue itself, and the ease and rapidity with which it could be removed from the cranium, that the others were abandoned for it. As a matter of fact, it was found possible to chloroform, remove the organ, and have the pieces of the practically living tissue in the hardening media within five minutes after the commencement of the narcosis. All the animals used were young, but full grown.

The following methods of hardening and staining were employed:

1. *a*. Flemming's fixation fluid, into which very small pieces of the cerebellar tissue were placed for from twenty-four to thirty-six hours at the room temperature of from 20° to 25° C.

b. The same process, except that the fluid was heated to 35° C. before the tissue was placed in it, and kept at that temperature for 24 hours in the warm chamber. Absolute alcohol after hardening for both series. The sections from *a* were the best.

The sections from this series were stained with safranin, generally for twenty-four hours, occasionally for several days; with carbolic-

acid fuchsin; and with my modification of Weigert's hematoxylin.[1] The safranin and osmic-hematoxylin preparations proved the most satisfactory of all the preparations. Unstained sections were also examined with comparatively good results.

2. *a.* Müller's fluid employed in the same way as the Flemming, namely, small pieces being dropped into the cold fluid and allowed to remain in it for six weeks, the fluid being frequently renewed in the meanwhile, and carefully kept from the light and air.

b. Cubes not more than half a centimetre square were placed in a large jar of fluid heated to 35° C, and the temperature maintained at that degree for ten days, the fluid being changed every second day. After the tenth day the jar was removed from the hot-air oven, and afterwards, as in the first method, the cubes were dehydrated in alcohol without previous washing in water.

The stains used with the Müller's fluid preparations were Weigert's hematoxylin, aqueous fuchsin, nigrosine, Weigert's säure fuchsin, borax carmine, alum-hematoxylin, eosin, and the carminate of soda staining *in toto* after Schultze.

The copper-hematoxylin, fuchsin, hematoxylin, and sodium carminate were the most useful. Combinations were frequently employed.

3. Disassociated preparations were also made from the fresh tissue, and after maceration in dilute Müller's fluid and osmic acid solutions, with fair results, principally to confirm certain points in sections. Some fresh preparations were stained in aqueous fuchsin.

4. The sublimate method with the quadruple stain of Gaule; also sections from sublimate stained with borax carmine. Neither presented any advantages over other methods.

5. Hardening in absolute alcohol, and after treatment according to the method of Nissl, proved extremely satisfactory for the cellular elements and glia tissue. Carmine tingeing also gave some very good specimens.

6. The sublimate method of Golgi, also as modified by Pal, produced some useful slides, though the terminal protoplasmic filaments of the Purkinje cells were brought out more satisfactorily by carbolic fuchsin with Flemming, and a modification of the Weigert copper stain, in which the metal is finely deposited upon the protoplasm,

[1] See Johns Hopkins Hospital Bulletin, No. XII, 1891.

the medullated tubes retaining their deep blue, while the cellular substance assumes a brown tint.

Imbedding was always done in celloidin, except in one or two instances; very often directly from the absolute alcohol into the ether-alcohol-celloidin mixture, which was found to answer perfectly. Sections were cut of varying thickness, from one down to less than half a space on the Schanze microtome. These sections were not confined to parallel and vertical cuts, but were made at a large number of angles to the surface, acute and oblique; and from these, some important items concerning the direction of nerve and other fibres are deduced. Serial sections were only made in a single instance, to determine the space between the Purkinje cell rows.

The Central Core and Medullated Fibres.

The central core of the lobules as it passes off from the deeper white masses into the leaflets presents no difference in the size or contour of its fibres from the ordinary structure of white medullated tissue. Now and then, scattered multipolar nerve cells are seen in it at the base of the folia; a few larger than those of the great cell layer of the cortex, and much resembling them in contour. These cells are isolated offshoots from the individual groups that always lie lower down in the white substance.

Scattered among the fibres are nuclei in about the same proportion as in the cerebral medullary matter. These nuclei are mainly oval, sometimes round; with Nissl's method very finely grained, with two or three larger particles of chromatin scattered irregularly through the nuclear substance. The oval nuclei show a small amount of protoplasm capping the end, and from this, delicate unstained fibrillæ, extending between the medullated fibres, may be seen. Ganglion cells, isomeric with the Purkinje, are met with, though but rarely, along the border of the granule layer.

As the core is traced toward the apex of the leaflet, fibres are seen to pass off from it at an oblique angle into the red-brown layer, until at the apex, at a distance of from two to three millimetres beneath the large cell layer, it spreads out like the sticks of a fan, with a somewhat regular arrangement.

The fibres may be easily separated into several classes, (1) a series of apparently unbranched, non-varicose, straight, medullated tubes, passing directly from the sides and apex of the core to the vicinity of the Purkinje cells. Some pass straight, others bend as they approach the "limitans interna"[1] to reach their individual cell. In sections perfectly plane to the surface, their connection with the Purkinje cells may often be demonstrated. As they enter the cell's capsule at its inferior pole, the medullated sheath is lost, or rather becomes directly continuous with the capsule of the cell, and is indistinguishable from it (Fig. 3). Rarely the fibre has the appearance of becoming non-medullated just before the capsule is reached, but such appearances are exceptional, and may be deceptive from the outspreading of the glia fibres from the capsule.

The axis cylinder is lost immediately after entering the protoplasm of the inferior pole, so far as I have been able to discover, but the capsule hides the true relation to some extent. Disassociated preparations from dilute Müller's fluid, unstained, or tinged with fuchsin, also show distinctly the axis cylinder entering the protoplasm, and rarely, a light striation may be discovered.

The second class also pass to or from the central core, generally by one of the so-called eosin cells of Dennissenko, and after continuing by it, branch indefinitely between the groups of granule cells; though through the densest of these groups the coarser fibres seldom seem to run; then continue by other eosin cells, and so on, forming an open network of a very complicated arrangement.

I have not been able to determine positively if all these fibres pass in close relation to an eosin cell, as they go to or from the core, before branching, but am of the opinion that some connect with other fibres before touching any of these bodies.

The fibres composing the network vary in size considerably, being seldom so coarse as the fibres of the first class, then passing in various gradations to the finest kind of medullated fibres, as fine as any demonstrable in the cerebral cortex. Even with the finest of them Weigert's reaction never fails, and I do not regard any of the fibres of the inter-granular plexus as being of non-medullated character. Indeed, very few non-medullated fibres can be determined in this zone, and all that are present are probably in direct relation with the

[1] See page 203.

scattered ganglion cells in this layer. At the point of juncture between fibres there is always a slight thickening of the medullary covering; generally the fibres are somewhat varicose.

Under the "limitans interna" branches shoot out from the plexus in numbers, and proceed vertically, or at a right angle into the molecular layer, where they rapidly attenuate, lose their medullated character, and for the most part are lost in the inferior and middle thirds, mainly in the neighborhood of certain small nerve cells lying in this region; while a minimum number continue toward the periphery, also losing their medulla and becoming reduced to the finest threads, when they can no longer be traced. Few continue beyond the middle of the layer. Numbers run for considerable distances in the band of tangential fibres, and then turn upward into the outermost layer.

The third class of medullated fibres are not very numerous. They proceed like the first class directly from the core through the granule layer, pass between the Purkinje cell bodies, then slanting considerably in their course, traverse a part of the barren zone, rapidly becoming attenuated and non-medullated, and disappear in the neighborhood of the before-mentioned small cells in fine terminations. Likewise these apparently do not branch.

Associating Fibres.—To the fibræ arciformes connecting the folia, no reference is made in this paper. Within each leaflet but one system that bears any approach to an association can be distinguished. This lies about and covers the capsule of the Purkinje cells with a thin layer of fibres, with a general arrangement and direction similar to that of the great cell row, but also around each cell's capsule a few fibres are wound like loose threads upon a ball. There is an apparent variety in the quantity of the fibres in each leaflet, but they are usually most numerous where the Purkinje cells are thickly set. There is also a difference in the breadth of the band in different leaflets. It never extends much beyond the inner limit of the "limitans interna"; on the other hand, its circular fibres may be met with as high as the central part of the molecular layer, but widely scattered. Generally its margin is within the outer limit of the inner third of this layer. Allowances are to be made for possible differences in staining. The fibres composing it are medium-sized medullated tubes, varying slightly in fineness, the medulla is double-contoured and stains easily blue-black with Weigert's hematoxylin.

In the outer zones of the molecular layer a few scattered medullated tubes may be seen, with a direction parallel or oblique to the pia; but they are insufficient in number to form any approach to a system.

Crossing the central core at right angles to the longitudinal fibres, medullated tubes may be found in inconsiderable numbers, running from the anastomosing fibres of one side to those of the other half of the granule zone.

The continuance of the straight, apparently unbranched fibre into the inferior pole of the Purkinje cell has been moderately definitely made out before, notably by Beevor, and the only uncertainty seems to be as to its manner of entering the pole. Fig. 3, drawn with the aid of a Zeiss immersion system, shows as nearly as possible the relation, though as before remarked the capsule hides it very often.

It is simply an hypothesis, but I am inclined to regard them as efferent fibres that run downwards to the cord, or more probably to nuclei of the medulla oblongata, their nerve impulses being continued further through a second set of cells; while those fibres that first lose their medullary sheath in the upper part of the inferior third of the molecular zone may be the afferent from the spinal cord. Many of them terminate near the small ganglion cells of the molecular layer, and probably have a different function from those coming from the Purkinje bodies. It is possible that the fibres of the third system may be in connection with those of the nerve cells of the molecular layer also, in which case they may also be considered as afferent. A portion of the fibres which arise from the nerve cells of the barren and granular zones may very possibly convey impulses to the cerebrum. The disappearance of a large part of the fibres passing from the anastomosing plexus into the barren zone, in close proximity to certain ganglionic bodies, leads to a suspicion of a direct relation between the two, and it is more than possible that the great majority of the fibres of the granular layer are centripetal-coursing.

It is difficult to understand the reason why an anastomosing network should have an association system at its outer edge, yet the only fibres that run into it in considerable numbers, and lie parallel with the band for any distance, belong to the plexus and may be traced directly into it.

As the band is generally best developed where the Purkinje cells are thickly set, and as all the fibres passing to and from the molecular layer pass through it, it may have the function of uniting, or rather transmitting the nervous impulses derived from the various kinds of cells in the cortex into one common channel, should necessity arise therefor.

The Granule Zone.—The limits of this layer are the least defined of any in the cerebellar cortex. Narrow at the base of the leaflets, it gradually spreads out to a breadth several times exceeding that of the inferior part. Likewise its round nuclei penetrate everywhere into the edges of the central white core, and to some extent along the borders of the molecular layer, though here the round nuclei are replaced in large measure by cells of another description.

Within these limits six different varieties of cellular bodies, exclusive of those appertaining to the blood-vessels, may be distinguished. The most numerous are the variously named, round, granule, or hematoxylin cells (Figs. 1, 2, 6), which were the distinguishing feature of the layer with the old carmine preparations, and with the addition of a small ring of protoplasm around each nucleus, make up more than half the substance of the zone. These round nuclei are a little smaller than those of the neuroglia in medullated tissues, but are unlike them in taking up much more of the hematoxylin, safranin, or magenta dyes, hence stand out more prominently in the field of the microscope. They are arranged with a certain definite grouping (Fig. 2), which is most pronounced at the apex of the leaflet, and shows spaces between each group, which are filled by the eosin cells, glia fibrillæ, and medullated tubes. The nuclei are always perfectly round, contain numerous small molecules deeply tinged; the smallest being arranged along the periphery, the larger somewhat more centrally, with always a single one of a greater size than the others, giving to a limited extent the appearance of a nucleolus. Around the nucleus is a diminutive ring of an apparently protoplasmic substance, easiest seen with the nigrosine stain, but at best difficult to distinguish in sections stained in any manner, as it generally remains untinged. In teased preparations, and in sections separated by pressure on the cover glass, it is, however, more clearly brought out. Threads extending from this protoplasmic ring are demonstrated with extreme difficulty, and I have only succeeded in a

few instances in convincing myself that they are present, then only in teased slides.

Widely but sparingly distributed through this layer are round, sometimes slightly oval nuclei, with a nuclear substance that does not take up any of the aniline dyes, but only shows a few minute molecules clustered along the margin, and an invariably distinct and well-tinged nucleolus lying in the centre (Figs. 2, 5). These clear nuclei are present in the spaces between the granule clumps, and are about double the size of the granules. I have never succeeded in isolating them with any protoplasm attached, nor distinguished fibrillæ proceeding from them. They are probably glia-tissue elements, having their prototypes among similar nuclei in the medullated and molecular regions.

The undoubted glia nuclei are separated into three different varieties, distinguished chiefly by their form and some difference in size. In the central and deeper regions of the zone they are infrequent, lying chiefly near the white fibres, for which their threads probably serve as supports. Close to the Purkinje cells they constantly increase in numbers, while the granule nuclei as constantly diminish, until between the lower half of the bodies of these cells (which is, however, properly in the limits of the molecular zone) but few of any kind of nuclei other than glia can be found.

Of the three varieties, a very small round nucleus, about the size of the granule nuclei, are to be seen in deeper parts, together with a few of an elongated oval form (Fig. 5), both lightly tinged and holding a few chromatin particles. In the same region may be seen nuclei tipped with a hood of protoplasm (Fig. 5), which sends off from its apex one prominent stem. This protoplasmic hood is apparently separate from the nucleus.

Along the margin of the "limitans interna" the round and pyramidal cells assume the importance of a double or triple, though irregular row, mainly with their broader bases directed inward; and from the latter nuclei come off very numerous fibrils ascending into the molecular layer (Fig. 1). At rather definite intervals a stronger thread ascends to the sub-pial limit, and is attached in the thickened glia on the periphery. In the central regions the glia fibrils, like their nuclei, are but poorly developed, the granule cell groups being so closely packed that with the other elements, inclusive of the

capillaries between them, there is but little room for any accessory fibre development.

But the aspect along the outer edge is widely different, the fibrillæ from the very numerous glia cells expand to a vast open network, wrapping the Purkinje bodies in a thick envelope, twisting and crossing between the nerve tubes, vessel plexus, and other cells in all directions. The name "limitans interna" has been given to this network of the neuroglia by Beevor.

Eosin Bodies.—The so-called eosin cells of Dennissenko form apparently a *unicum* in the histology of the central nervous system. They consist of spindle, triangular (which largely predominate, and show a triangular aspect both on vertical and horizontal section), and irregularly quadrate bodies; confined entirely between the extreme inner limit of the "limitans interna" and the central core, into which they press to some extent, depending upon the density or rarefaction of the nerve fibres. They are somewhat more numerous toward the apex and central regions of the granule zone, but present few modifications in size or general appearance in its different portions. For actual examination they stain only with the Flemming-copper-hematoxylin, and the Weigert so modified to produce a copper precipitate. Eosin, nigrosine, and fuchsin also tinge them, but with the exception of the last the definition is far from perfect. Something may be done with teased preparations, especially after they have been some time in dilute osmic acid solution.

The eosin bodies are arranged in irregular collections, with rows or masses of granule cells separating them; and wherever the nerve fibres of the anastomosing plexus lie thickest, there also the eosin cells are best developed. Their size varies from about $13 \times 10\mu$ up to $18 \times 21\mu$ for the triangular; $13 \times 15\mu$ to $33 \times 19\mu$ for the largest and smallest spindle, and about $9 \times 8\mu$ for the irregularly shaped ones. The thickness is about the same as the breadth. The cell body is formed in large proportion of coarsely granular particles, with here and there irregularly placed larger ones (Figs. 1, 4), both round and rod-like in shape, all imbedded in a small amount of a homogeneous matrix, which can only be tinged effectually after treatment with osmic acid (carbol-fuchsin, and Flemming-hematoxylin). No nucleus or necleolus is anywhere visible in any of them, the closest approach to it being in unstained or safranin preparations,

where there occasionally appears a vacuolated appearance toward the centre of the body, which resolves, after osmic acid treatment, into one or more of the largest granules lying close together.

The relation of the anastomosing nerve fibres to these granular bodies is peculiar and interesting. With säure and carbolic fuchsin the impression is given that the nerve tube enters the body, loses its medullated sheath, and regains it beyond; but this is not confirmed by either the Flemming or Weigert hematoxylin stains, nor by the safranin method of Adamkiewitz. The nerve fibres are now seen to run in close approximation to the granular body (Fig. 4), over it, or along its margin, but do not directly enter. Occasionally there is the appearance as if a few of the finer granules were thrown over the medullated sheath, but this is rare, and may be deceptive.

Numbers of all, but especially the triangular forms, are surrounded on two or even on all three sides by the medullated tubes, so as to present the appearance of a blue-black triangle inclosing a brown centre (copper precipitate preparation). The fibres after passing beyond the body again join the network and proceed to branch indefinitely. Nowhere can any connection between the axis cylinder and the granular cells be demonstrated, nor does the medullated sheath lose its contour. Fibres also cross the long and transverse diameter of these bodies in all directions, but apparently only touching their surface, sometimes even branching as they reach the margin, then sending a double fibre over the surface to finally disappear in the network.

Very fine-grained processes can be seen extending from the poles of the cells, in sharp contrast by their brown color with the medullated fibres, their terminations disappearing between the rows of the hematoxylin nuclei. Besides these rami there may be seen exceedingly fine threads coming apparently from the body, which, on first examination, also look like extensions, but with immersion systems they are seen to have an exceedingly thin layer of blue medullated substance around a darker centre, and when they can be traced any distance join fibres of the branching system. Accordingly, they probably rise from layers beneath the section, to the body, then pass on a level with it to connect with other fibres of the same system. Around isolated granular cells a number of unstained, exceedingly fine filaments may be distinguished by their refraction, but these

are probably derived from the neuroglia, which is better developed in their neighborhood than elsewhere in the granule zone.

Throughout this zone a considerable number of multipolar, bipolar, and pyramidal-shaped nerve cells are to be found scattered here and there. They have well developed nuclei, nucleoli, and processes, the last soon becoming lost among the granule cells. There is also an extremely rare, very large, irregularly-shaped nerve cell, with an excessively large, clear, oval nucleus, with few chromatin particles in it and a bright small nucleolus. The protoplasm and extensions are with any method of staining very ill defined (Fig. 6). There is no discoverable relation of any of these cells to nerve fibres, though, of course, it must exist.

The question whether the granules are the nuclei of nerve or glia cells has been for many years a much combated point. My researches have led me to place them, like certain other small cells in the posterior horns of the spinal cord, among nerve formations, and upon the following grounds: In certain diseases of the cerebellum they, in common with the ordinary fragile nerve elements, atrophy, and even disappear entirely, while the neuroglia cells usually remain and are increased in quantity. In a recent case of the so-called gliosis of the cerebellum, a portion of the organ was hardened in Flemming, and stained in safranin and osmium-copper-hematoxylin.

The whole granule layer viewed with a low power appeared less dense than usual; when examined with an immersion system, the granules were seen to be wider apart, looked more coarsely grained than normal, while among the groups were ill-stained fragments of the cells, apparently undergoing a disintegrative process. There was, moreover, a difference in the absorption of the dye by the nuclear substance generally. A distinct disappearance of the medullated tubes and eosin cells of the layer was also quite evident; the increased space being filled in by an abundant growth of rather coarse fibres, while the nuclei of the neuroglia stood out quite prominently. In complete sclerotic degeneration of a cerebellar leaflet or lobule from arterial disease, the granules, in common with the more prominent nerve elements, completely disappear.

The presence of an exceedingly small amount of ordinary neuroglia tissues in the granule layer has been previously mentioned by Obersteiner and others, who give no detailed description. This small proportion in the central parts of the layer has very recently been demonstrated by Weigert, by means of his new method of staining the neuroglia tissue solely.[1]

The eosin cells, the most peculiar and striking feature of this layer, are not the least explicable element of this most intricate organ. They possess most of the attributes of a cell—and a nerve cell at that—protoplasm coarsely granular, prolongations, etc., but the nucleus is wanting, or at least cannot be demonstrated by any known method of staining, or be discovered in the unstained cell; yet if they are nerve cells it is a necessity that they have a nucleus, as no exception is known in the animal body. It is possible that the nucleus is very small, and may be hidden in the thick-set coarse granules that cover and overshadow it. If nerve cells they be, they are probably subsidiary to cells of higher function, the Purkinje for instance, and possess a connection with the fibres of the anastomosing plexus through their fine protoplasmic processes, that are utterly impossible to trace for any considerable distance from the cell body, through the thickly intervening granules.

Their intimate relation to the medullated fibres is singular, and does not exist elsewhere in the nervous system. There are places in the layer where medullated fibres are scattered, in which there are no eosin cells, but where the eosin bodies are numerous the medullated fibres multiply in numbers, seemingly not on account of an origin from these bodies, but because they seem to be placed along their paths.

Why should they enter into such intimate contact with these cells, curving along their rounded borders, or passing in close contact over them, unless they received some impulse from them? Yet the medullated sheath certainly continues uninterruptedly by the bodies; and the axis cylinder, so far as can be determined, does not divert any fibrillæ at the point of closest relation, nor is there any thickening of the medullary covering, as is seen elsewhere when the fibres branch in the plexus. Their complete atrophy in common with the undoubted nerve elements helps to classify them among the same structures.

[1] Anat. Anzeiger, No. 19, 1890.

The Great Cell Row and Molecular Layer.

The Purkinje cells form the most prominent connecting link between the granule and barren layers of the cerebellum. They are flask-shaped protoplasmic bodies, with their central prolongations passing through the "limitans interna," to be united with one of the radiating coarse medullated fibres that enter the central core. Best developed at the apex of the leaflet, they become somewhat less numerous in the depths of the furrows. In size they are about the same as in man, measuring 38μ in their long diameter, 25μ in their transverse, and from 20 to 23μ in thickness. The rows of cells are not perfectly parallel, and in cross sections are arranged a little irregularly in each line. Nearly surrounding them on all sides are the numerous glia cells, from which their capsule of the finest felt-work of fibres is derived. This enveloping is very clearly and distinctly seen in Flemming and copper-precipitate preparations. Where the medullated fibre enters the inferior portion of the central pole, its sheath immediately enters into and becomes a part of it, or at least is not distinguishable from it, staining the same hue, and the axis cylinder is lost in the cellular protoplasm, which for some distance is fibrillated. At this point of entrance the capsule is thickest and more dense than at the sides and apex (Fig. 3). The capsule extends rather further down on the central pole than is drawn in the figure. This capsule is continued upward on the chief prolongations, gradually diminishing in thickness, until it becomes over the medium-sized processes a microscopically structureless membrane, supported by, and in close relation with the neuroglia tissue and perivascular spaces of the smallest vessels in the molecular layer, and is doubtless a portion of the glia formation.[1] This enveloping of the peripheral prolongations is distinct in chrome and Flemming preparations (carbol-fuchsin, safranin, also in unstained sections), less easily demonstrated in alcohol ones.

The encapsuling of the Purkinje cell gives a lymph space which, for the barren layer taken as a whole, is of stupendous extent. Horizontal sections of the cortex in the inferior third of the zone also show the cell branches with a clear ring around them (Müller-car-

[1] The finer portion of the capsule may possibly be derived from the hyaline sheath of the vessels; the thicker portion is certainly made up from fibrillæ.

mine-nigrosine). Mixed with the glia fibres surrounding the body of the cell are numbers of very fine naked axis-cylinder threads, apparently coming from the molecular layer to reach the cell.

The processes that extend peripheralwards give a variety to the appearance of these bodies. Some, and that the majority, have a single thick process, which usually leaves the body perpendicularly to the periphery; while others have a thick process that almost immediately divides; and there is a third variety in which the arms leave the cell at opposite angles (Fig. 1).

With unstained, and with most of the usual methods of tingeing, the body of the cell appears finely granulated, with a certain concentric arrangement, which gradually disappears at the upper pole, beyond which the prolongation usually shows a light striated appearance, which grows less and less distinct, disappearing entirely in the finer branches. The inferior pole of the cell has also sometimes a striated arrangement. Nissl's stain gives a number of coarse granules among the finer ones of the protoplasm. The cell body never contains any pigment deposit.

The nuclei are invariably clear, round or slightly oval, and hold a number of round bright-colored particles that with Flemming are often rod or threadlike, giving something of a radiation around the nucleolus. This nucleolus is rounded, very often slightly irregular, colors brightly, and is finely grained.

From the protoplasmic processes of the preceding paragraph thick branches arise, that again and again subdivide, almost invariably at right angles to the periphery, until they become filaments of tenuity, and form a feltwork inconceivable in its magnitude and fineness.

From the first branches that come off from the main stems at an angle of about 180° to the surface, filaments pass upward and downwards, the latter filling the space to some extent between the thick rectangular processes, and the small nerve, and pear-shaped glia nuclei. The former pass upward, in distance commensurate with their thickness, until they become too fine to be followed. Like the upper branches of a tree, denser and denser does the feltwork become as the periphery is approached (plexus is not applicable because there are no anastomoses to be discovered between filaments; this is absolutely true for the coarse processes, but the terminal filaments from their very tenuity preclude the possibility of certainty).

Many of the finest, like those in lower planes, turn downward in the process of division, but a large number approach the surface, still dividing, until they almost reach the sub-pial limit, where they apparently end. Doubtless this is the fact with many of them, but in sections taken at a somewhat oblique angle to the surface, a portion may be seen to turn at a different angle from which they arose, pass a minute distance nearly horizontally, and descend again, to be lost before reaching the middle third of the layer. Numerous darkly colored points (Flemming-hematoxylin, copper precipitate) at the ends of many of the filaments attest that a considerable portion through the whole layer change the angle of their course, their divided ends showing more deeply colored than the other part of the process, because it is now turned toward the objective and the observer is looking through a deeper bit of protoplasm—hence the intense stain.

Variations and individual differences are of course not wanting in the manner of branching. One of the most common is for a cell to throw two or three thick primary branches upward past the middle third, but comparatively few filaments being given off at first by them; then past the centre, or even higher in the layer they divide interminably, or still proceeding upward to within 40 to 50μ of the surface, they throw off a fine spray of rarefied filaments, that, after approaching nearer to the "limitans externa" at an oblique angle, again pass off, descend, and are lost. Another peculiarity of the Purkinje cells which has been previously pointed out is that they spread out only in one direction. If the section is made in the long diameter of the convolution, yet perpendicular to the surface, it will be seen that the branches extend but little beyond the thickness of the cell itself; or, differently expressed, the rows are set like well trimmed osage-orange hedges planted closely together, and only capable of free expansion in a single direction—upward, and within the free space between their bodies,—though like the trees of the hedge there is much interlocking and crossing of the branches.

Beyond the great cell layer, extending as high as the limit of the inferior third of the molecular layer, lie considerable numbers of small, rounded and angular multipolar cells, with distinct protoplasm, large nuclei containing coarse grains, and well developed prolongations. The largest of them are $18 \times 12\mu$ and are undoubtedly nerve

cells. In their neighborhood, but peripherally, are numbers of still smaller cells with a relatively large nucleus, and ill-defined though present protoplasm. These do not belong to any of the glia types, and are almost certainly, like their larger neighbors, nerve cells.

It is in the immediate vicinity of both of these cell varieties that the nerve fibres that cross the "limitans interna" pass and disappear; no connection is to be ascertained between them, yet from their being perfectly visible until they reach the neighborhood of the nerve bodies, then rapidly attenuate and are lost, a connection between the two is more than probable. A few fibres continue on beyond the inferior third of the zone, but they soon become non-medullated and disappear; but four medullated fibres in some 50 Weigert sections continued as far as the sub-pial periphery or to its vicinity. Some non-medullated fibres in inconsiderable numbers may occasionally be seen lying along the outer border of the band of tangential fibres, among the Purkinje cell extensions (safranin).

The nuclei of the neuroglia in the barren layer are distributed throughout its central and mid-regions in rather less number than in the white tissue. Toward the pia, nuclei become less and less obvious, especially upon the vertex of the leaflet, where in places for comparatively large areas they are entirely absent; the entire space being filled, almost exclusively, by the ultimate ramifications of the Purkinje cells, capillaries, and supporting trabeculæ of the pial connective tissue framework. The oval form predominates among these nuclei. Very many of their poles, with a little granulated protoplasm attached, are directed vertically, and send out fine fibrillæ upward and downward, crossing others that come from less frequent cells lying horizontally. Some of these glia fibrils are sufficiently coarse to allow of being traced to the external limiting membrane, and to the region of the pyramidal nuclei around the great cell row. All of these fibres, together with the capillaries, help to fill up the small remaining space between the Purkinje cell prolongations, and support the soft substance.

The "Limitans Externa" and Pial Connections.

On the outermost border of the leaflets subjacent to the pia is a condensation of substance marked by a deeper staining with certain reagents, notably nigrosine-carmine. With absolute alcohol (Nissl)

and Flemming sections, this condensation is seen to be owing to a feltwork of fine glia fibrils, which are derived from a number of oval and pyramidal nuclei here situated. By no other process of hardening and staining can these nuclei be made sufficiently conspicuous to be clearly seen. They vary greatly in number, the row in some spots being quite thickly set, in others very widely scattered (Fig. 7). From a part of these nuclei the feltwork is derived, while others contribute many fibrillæ that descend, some to be lost in the higher portions of the layer, some to descend as far as the pear-nuclei row and terminate among them in the glia network. Between them can be seen, occasionally, triangularly-shaped bodies, from which descend stouter threads than from the pyramidal nuclei, which are continued directly, waving through the interposed elements, down to the pear-shaped and round cells of the Purkinje cell row, from which they apparently arise, and have their termination in these triangular bases, which are only a thickening of the glia substance.

Outwardly from this limiting membrane lies a sub-pial lymph space of considerable magnitude, crossed by rather thick connective tissue threads, that arise without much thickening at the point of origin, from the innermost layer of the vascular pia, crossing the lymph space, pass directly through the outer limiting membrane, and then through the molecular layer to the glia cell layer at its base, among whose fibres they also terminate. In the substance of the layer they are only to be distinguished by their larger calibre from the fibres arising from the glia cells (Fig. 7).

The arrangement of the outer limiting membrane and the pial trabeculæ is best seen between the lobules where the component parts are more spread out than on the surface; especially is this true of the trabeculæ which in the depths of the furrows arise in such quantities as to form a brush-like figure, giving the most efficient support to the fragile nerve tissues of the cerebellar cortex.

The number of nerve fibres entering into or passing from the molecular layer precludes the possibility of the termination of every large branch of a Purkinje cell becoming ultimately a nerve fibre, as has been supposed, and in fact there is no necessity to believe that more than one medullated nerve fibre can arise from a single

cell, either physiological or histological, every evidence being to the contrary; and all the centripetal and centrifugal coursing fibres must have their beginning in a single cell, their ending admitting of some doubt. However, there are sufficient nerve cells in the molecular and Purkinje cell layers to accommodate quite the number of fibres that are to be seen.

The nerve elements of the cerebellum viewed as a unit bespeak a sensory and not a motor organ. The great cells of Purkinje have a closer likeness to those of Clarke's columns, which are supposed to preside over a department of sensation, than to any other bodies in the nervous system. The round granules correspond in some measure to the granule layers of the retina, and the small cells of the molecular layer are like those in the posterior horns of the cord; all correspond with conceived ideas of sensory cells, and only a few multipolar bodies have any approach in appearance to motor cells.

Lesions of the cerebellum, with the exception of the middle lobe, are without objective symptoms. It is conceivable that an involvement of the cortex of the middle lobe may also be symptomless, and that the true reason of the perversion of the sense of equilibrium in disease of this part is because the multiform crossings of fibres to and fro from one part of the organ to another are intersected, and that the symptoms arise, not from lesion of the cortex of the vermis or middle lobe, but from the destruction of the function of these crossing fibres, which then produces a loss of function, not of the middle lobe solely, but of the whole cerebellum.

In the whole series of sections from all parts of the organ no essential difference was to be discovered between any of the different regions.

March, 1891.

Explanation of the Plate.

Fig. 1. Skeleton drawing of one-half a cerebellar leaflet, built up from safranin, carmine, Weigert's hematoxylin, Flemming-Weigert hematoxylin, carbolic fuchsin, Nissl's magenta, and sodium-carminate sections. *a*, central core of white medullated fibres; *a*1, glia nuclei among them; *b*, layer of granule or hematoxylin cells (all the eosin cells are not represented in this portion of the drawing); *c*, the granule layer with the eosin cells, straight nerve fibres running to

the Purkinje cells, and the anastomosing fibre plexus; *d*, *d*, eosin cells; *e*, the fibre plexus; *f*, *f*, the straight fibres running through the granule layer, those passing directly into the molecular layer are not represented; *g*, tangential fibres at the outer edge of the granule layer; *h*, *h*, the Purkinje bodies with their outspreading branches; *h*1, *h*2, turning of the terminal filaments beneath the pia; *i*, the pia covering the surface of the leaflet; *k*, nuclei in the sub-pial space; *l*, *l*, the small nerve cells in the inferior third of the molecular layer; *m*, fibres radiating out of the granule layer; *n*, nerve cells in the granule layer; *o*, the row of pear and round nuclei at the outer edge of the granule layer; *o*1, the Purkinje cell space, capsule not drawn; *p*, *p*, glia elements of the molecular layer; *o*, *o*, fibrillæ passing off from the pear-shaped cells attached to the limitans externa; *r*, supposed glia nucleus in the granule layer; *s*, the limitans externa; *t*, granule nuclei. Zeiss apochromatic immersion 1–12, ocular 4, reduced.

Fig. 2. The grouping of the granule cells, Nissl's magenta. *a*, *a*, granule cells; *b*, larger clear nucleus belonging to the glia elements. The clear spaces between the granules are filled in by eosin cells, glia cells and medullated fibres.

Fig. 3. A Purkinje cell divided through its centre to show the relation of the capsule and the cell body. Capsule *a*, *b*, from copper precipitate, nigrosine and safranin; medullated fibres and axis cylinder from Weigert, Flemming-Weigert, and safranin; cell body from carmine and magenta; striations from nigrosine; nucleus and nucleolus from magenta. *c*, glia nuclei, the threads from whose bodies form the capsule, from magenta, safranin, and carbol-fuchsin preparations. *c*, *c*, medullated fibres.

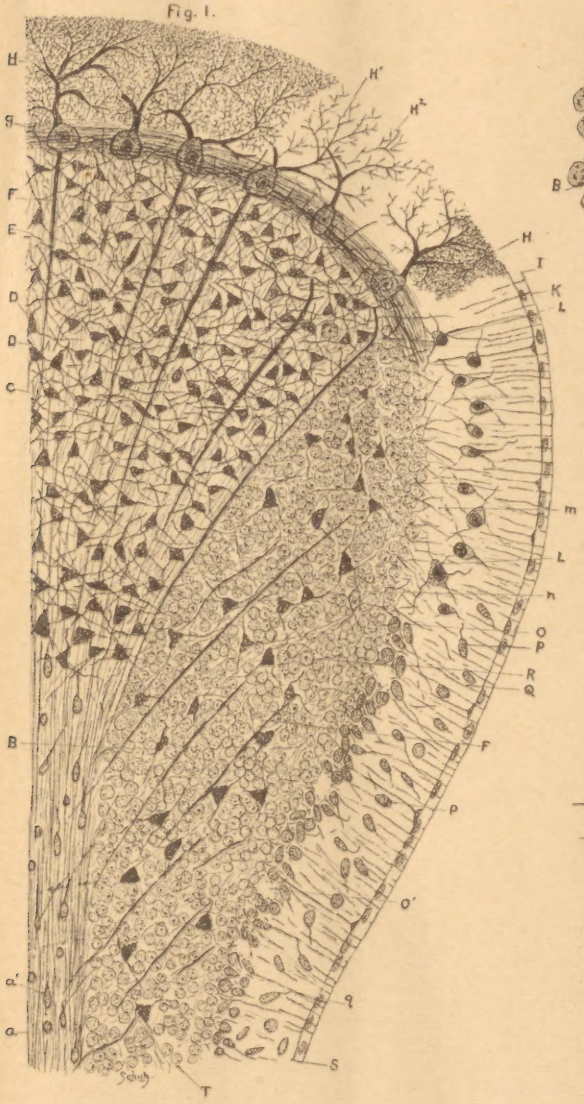
Fig. 4. Eosin cells, granule layer with the medullated nerve fibres, *a*, *a*, coursing over and by them, anastomosing and branching at different points; *b*, coarser medullated fibre; *c*, large granules in the body of the eosin cell. Copper precipitate preparations.

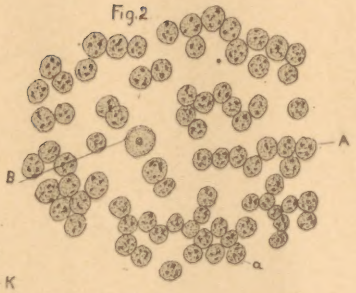
Fig. 5. 1, 2, 3, 4, 5, small multipolar and bipolar cells from the inner third of the molecular layer; 6, nerve cell with large nucleus and ill-defined protoplasm and extensions; from the granule layer: 1, carmine-nigrosine; 2, 3, aqueous fuchsin; 5, 6, magenta.

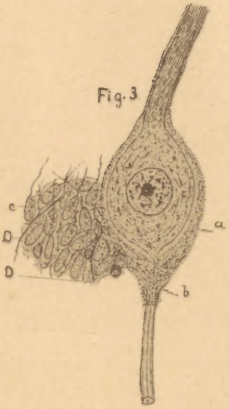
Fig. 6. Nuclei from the granule layer, showing the arrangement of the chromatin particles. 1, 2, 3, 4, neuroglia nuclei; 5, 6, nuclei

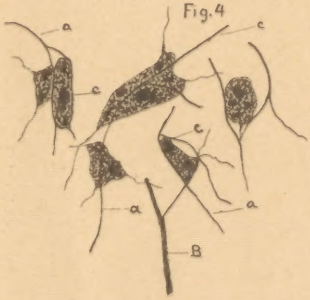
of the hematoxylin cells, showing a minute ring of protoplasm around the nuclear substance. Magenta, nigrosine.

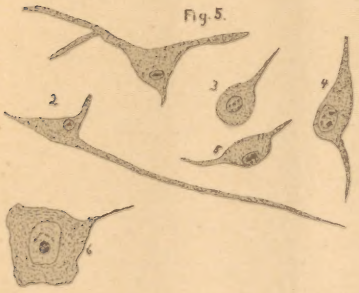
FIG. 7. The cerebellar pia with its connections. *a*, the inner layer of the pia, a few nuclei are seen in the meshes; *b*, the lymph space between the pia and limitans externa, with the meshwork of fibres and oval nuclei; *c*, the "limitans externa," made up of fine threads arising from the oblong and round nuclei; *d*, *e*, stout fibres arising from the pia, descending entirely through the breadth of the molecular layer. Magenta and carbol-fuchsin sections. All the drawings were made with the aid of a Zeiss microscope, ocular 4, apochromatic immersion 1–12.

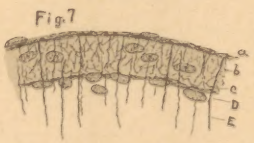












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CEREBELLAR CORTEX.